Site-Specific Incorporation of Tryptophan Analogs into Recombinant Proteins in Bacterial Cells

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Supporting Information (SI)

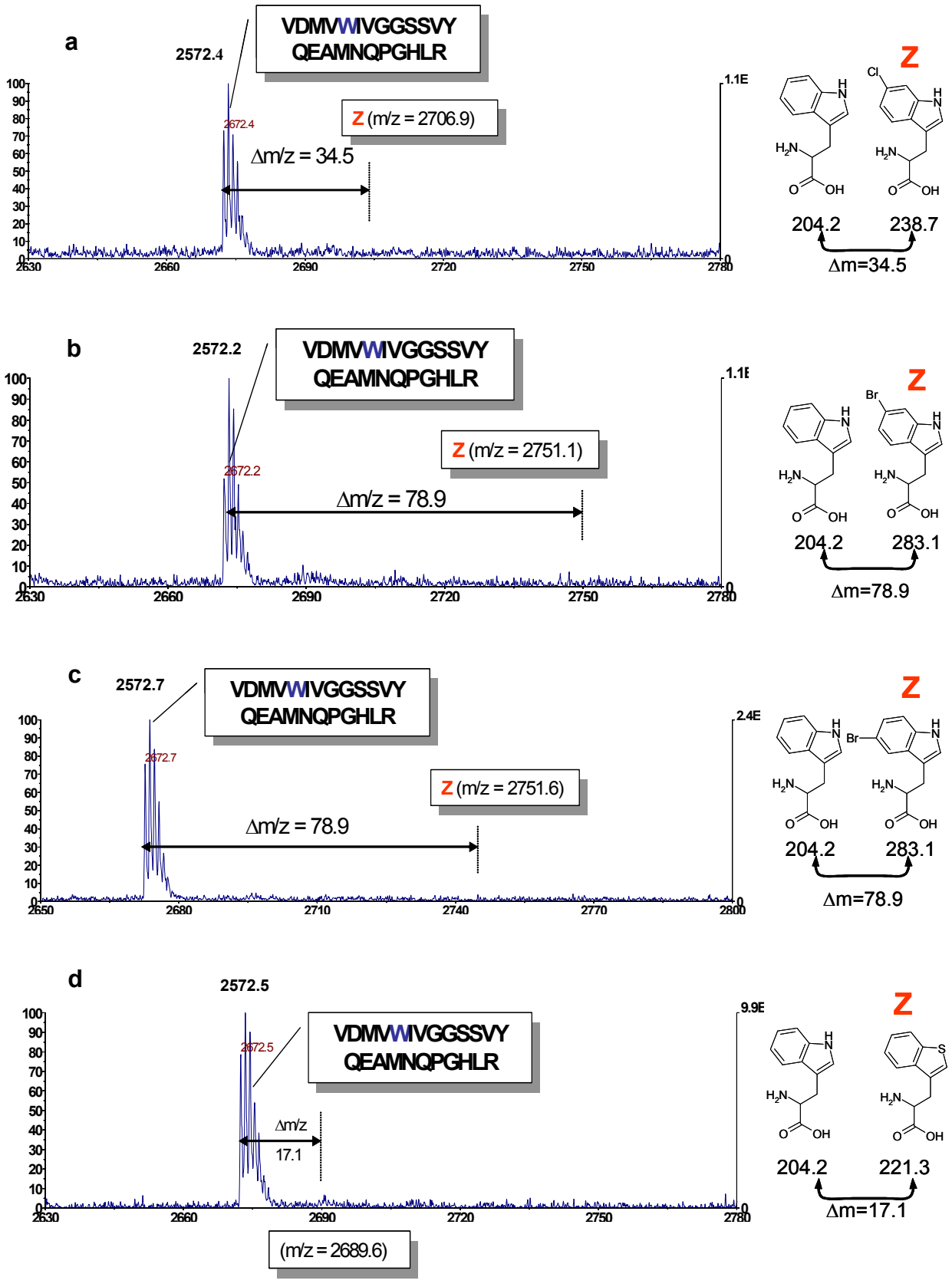
a
2572.4
VDMVWIVGGSSVY
QEAMNQPGHLR
Z (m/z = 2706.9)
Δm/z = 34.5
Z
204.2
238.7
Δm=34.5
b
2572.2
VDMVWIVGGSSVY
QEAMNQPGHLR
Z (m/z = 2751.1)
Δm/z = 78.9
Z
204.2
283.1
Δm=78.9
c
2572.7
VDMVWIVGGSSVY
QEAMNQPGHLR
Z (m/z = 2751.6)
Δm/z = 78.9
Z
204.2
283.1
Δm=78.9
d
2572.5
VDMVWIVGGSSVY
QEAMNQPGHLR
Δm/z
17.1
(m/z = 2689.6)
Z
204.2
221.3
Δm=17.1

Figure S1. Incorporation of Trp analogs at UGG Trp codons was investigated by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) analysis. A Phe/Trp double auxotrophic *E. coli* strain (AN67 obtained from CGSC at Yale) was outfitted with yPheRS (T415G) and ytRNA$^{Phe}_{CUA}$. mDHFR_38Am variants were expressed in culture media supplemented with 18 amino acids (at 25 mg/L), 30 μM Phe, one of Trp analogs and the indicated concentrations of Trp. a: 3 mM 6ClW and 10 μM Trp; b: 3 mM 6BrW and 30 μM Trp; c: 3 mM 5ClW and 10 μM Trp; d: 3 mM BT and 10 μM Trp. Trypsin digestion of purified mDHFR_38Am variants yielded a peptide containing a Trp codon (residue 116-139, VDMVW$_{UGG}$IVGGSSVYQEAMNQPGHLR). Insertion of the analog in response to the UGG codon would have given rise to a predictable mass shift in the spectrum of this peptide. No mass-shifted signals were detected, consistent with analog insertion in response to the amber codon.

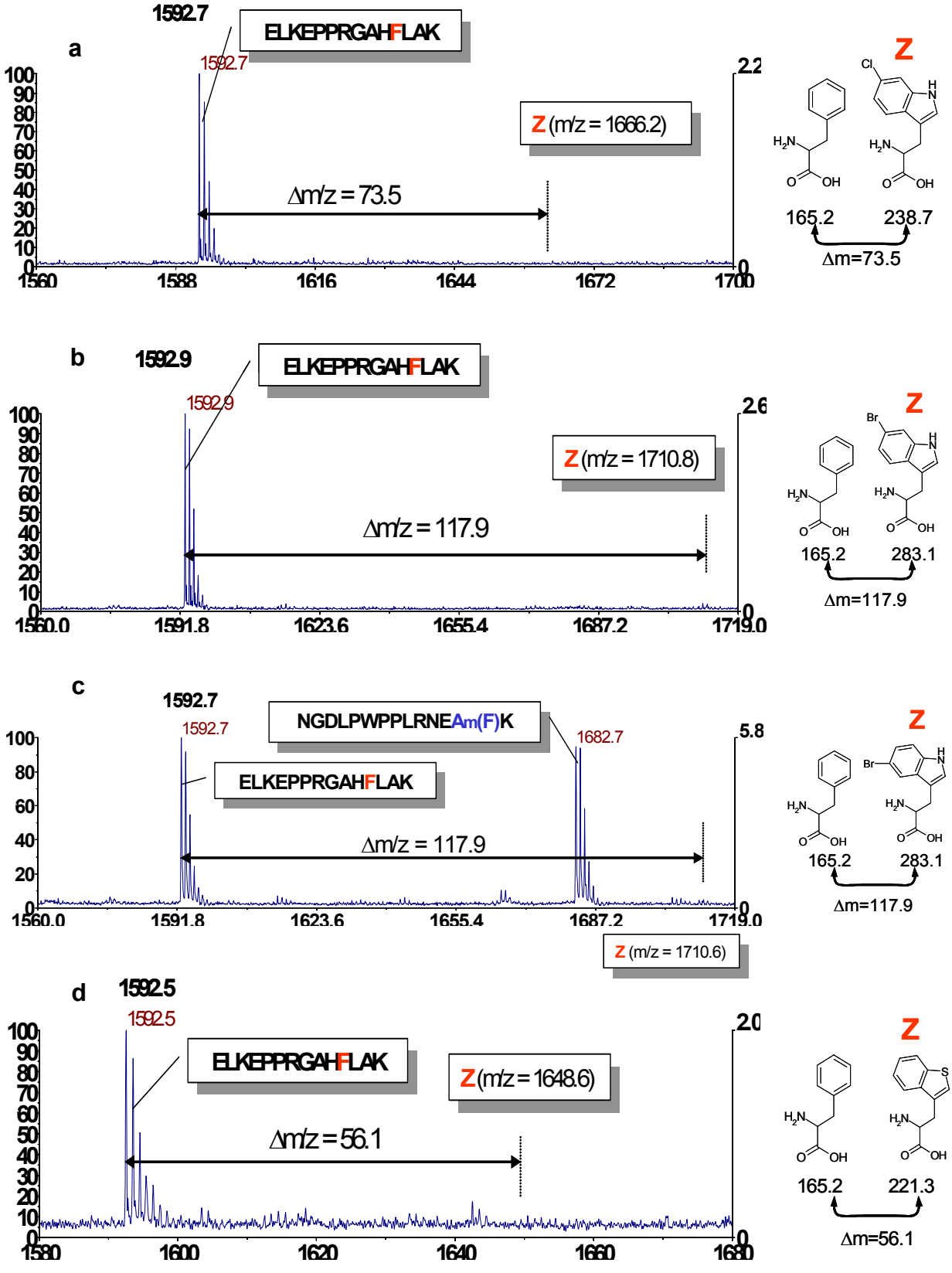
a
1592.7
ELKEPPRGAHFLAK
1592.7
Z (m/z = 1666.2)
Δm/z = 73.5
Z
165.2
238.7
Δm=73.5
b
1592.9
ELKEPPRGAHFLAK
1592.9
Z (m/z = 1710.8)
Δm/z = 117.9
Z
165.2
283.1
Δm=117.9
c
1592.7
1592.7
NGDLPWPPLRNEAm(F)K
1682.7
ELKEPPRGAHFLAK
Δm/z = 117.9
Z
165.2
283.1
Δm=117.9
Z (m/z = 1710.6)
d
1592.5
1592.5
ELKEPPRGAHFLAK
Z (m/z = 1648.6)
Δm/z = 56.1
Z
165.2
221.3
Δm=56.1

Figure S2. Incorporation of Trp analogs at Phe codons was investigated by MALDI-MS analysis. A Phe/Trp double auxotrophic *E. coli* strain (AFW) was outfitted with yPheRS (T415G) and $ytRNA^{Phe}_{CUA\_UG}$. mDHFR_38Am variants were expressed in culture media supplemented with 18 amino acids (at 25 mg/L), one of Trp analogs and the indicated concentrations of Phe and Trp. a: 3.0 mM 6ClW, 0.03 mM Phe and 0.01 mM Trp; b: 3.0 mM 6BrW, 0.015 mM Phe and 0.005 mM Trp; c: 3.0 mM 5BrW, 0.03 mM Phe and 0.01 mM Trp; d: 3.0 mM BT, 0.01 mM Phe and 0.0025 mM Trp. Trypsin digestion of purified mDHFR_38Am variants yielded a peptide containing a Phe codon (residue 85-98, ELKEPPRGAHFLAK). Insertion of the analog in response to the Phe codon would have given rise to a predictable mass shift in the spectrum of this peptide. No mass-shifted signals were detected, consistent with analog insertion in response to the amber codon. In case of c, peptide 38 containing Phe at an amber site (NGDLPWPPLRNEAm(F)K) was clearly detected in the spectrum.